STUDIES ON THE PHYSIOLOGICAL VARIATION BETWEEN TROPICAL AND TEMPERATE ZONE FIDDLER CRABS OF THE GENUS UCA. III. THE INFLUENCE OF TEMPERATURE ACCLIMATION ON OXYGEN CONSUMPTION OF WHOLE ORGANISMS [1]

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As organisms extend their distributional limits, they are faced with new environmental stresses. Through the mechanism of natural selection, populations of animals have arisen which show varying degrees of physiological adaptation. Review papers by Prosser (1955) and Bullock (1955) have excellent discussions of many aspects of this subject.

The present series of papers deals with physiological variation between temperate and tropical zone species of fiddler crabs (genus *Uca*). Results of the first paper (Vernberg and Tashian, 1959) demonstrated the marked difference in thermal death limits, especially at low temperatures, of latitudinally isolated populations of fiddler crabs. The 50% mortality level reached after 30–40 minutes at 7° C. for *Uca rapax* was characteristic of the other fiddler crabs studied in Jamaica, The West Indies, while temperate zone species from North Carolina lived for weeks at this temperature. Acclimation to reduced temperature (15° C.) had little effect on the response of tropical species but greatly increased the survival time of temperate zone forms. The second paper (Vernberg, 1959) dealt with the oxygen consumption of fiddler crabs as influenced by temperature, season, size and starvation. The seasonal variation in rates of oxygen consumption observed in *U. pugnax* from North Carolina, but not in the similar sized crab, *U. rapax* from Jamaica, suggested that temperate zone crabs possessed a labile respiratory mechanism while the response of tropical species is fixed within narrow limits.

The importance of temperature acclimation studies in understanding the metabolism of latitudinally isolated populations has been stressed for some time. Therefore the present investigation was undertaken to study in more detail the role of acclimation in determining metabolic differences between tropical and temperate

zone species. Since this study began, papers on crab respiration by Démeusy (1957), Roberts (1957) and Teal (1959) have further emphasized the need for additional work on this problem.

Materials and Methods

In the present paper two species of *Uca* were selected, *Uca pugnax* (Smith) from North Carolina and *Uca rapax* (Smith) from Jamaica and Florida. These particular species were chosen for a number of reasons: both species are closely related; until recently (Tashian and Vernberg, 1958), both species were considered races belonging to one species, the southern form being *U. pugnax rapax* and the northern race *U. pugnax pugnax*. Populations of these two species are found on the coast from Massachusetts to Brazil with a zone of overlap along the northeast coast of Florida. Thus they represent an excellent continuous series for a comparative study. Both species not only are available in great numbers, but they are easily maintained under laboratory conditions. Results of acclimation studies could be correlated with detailed metabolic data already available on these two species (Vernberg, 1959).

Experimental studies on fiddler crabs from North Carolina and Florida were conducted either at the Duke University Marine Laboratory or at Duke University, and the tropical species was studied at the University College of the West Indies, Jamaica.

The method of determining oxygen consumption, as well as the procedure for the laboratory care of animals, was described in a preceding paper (Vernberg, 1959). All results are expressed as cu. mm. oxygen consumed/minute/gram of animal (wet weight). Preliminary studies showed that the time interval necessary for thermal equilibration and for the rate of oxygen uptake to reach a somewhat steady level varied inversely with temperature. Only oxygen consumption data which were relatively stable over a period of time were used. An attempt was made to minimize variation due to rhythmic daily fluctuations by making an equal number of determinations in the afternoon and the morning. No attempt was made to correct for possible tidal influence on metabolism. Although the determinations were made at different times of the year, the recent thermal history of animals from North Carolina and Jamaica was the same in that their habitat and laboratory temperatures were alike. Work at Beaufort extended from June to September, while studies in Jamaica were from October, 1957, to April, 1958. Animals from Florida were collected in October and November and maintained in the laboratory until needed.

Animals subjected to 15° C. for various periods of time are referred to as cold-acclimated or "cold" animals, while animals maintained at room temperature (22–27°) will be called warm-acclimated or "room" animals.

Results

Uca pugnax from Beaufort, North Carolina

The metabolic response of 21 warm-acclimated *U. pugnax* was determined at 27° C. and then these animals were kept at 15° for 21 days. Throughout the period

of exposure to reduced temperature the rate of oxygen consumption was re-determined at 27° C. at specific intervals of time. Another group of animals, which were maintained in a starved condition at room temperature, were used as a control. Thus the per cent change in metabolic rate shown in Figure 1 represents shifts in rate of oxygen consumption relative to control animals as 0% change.
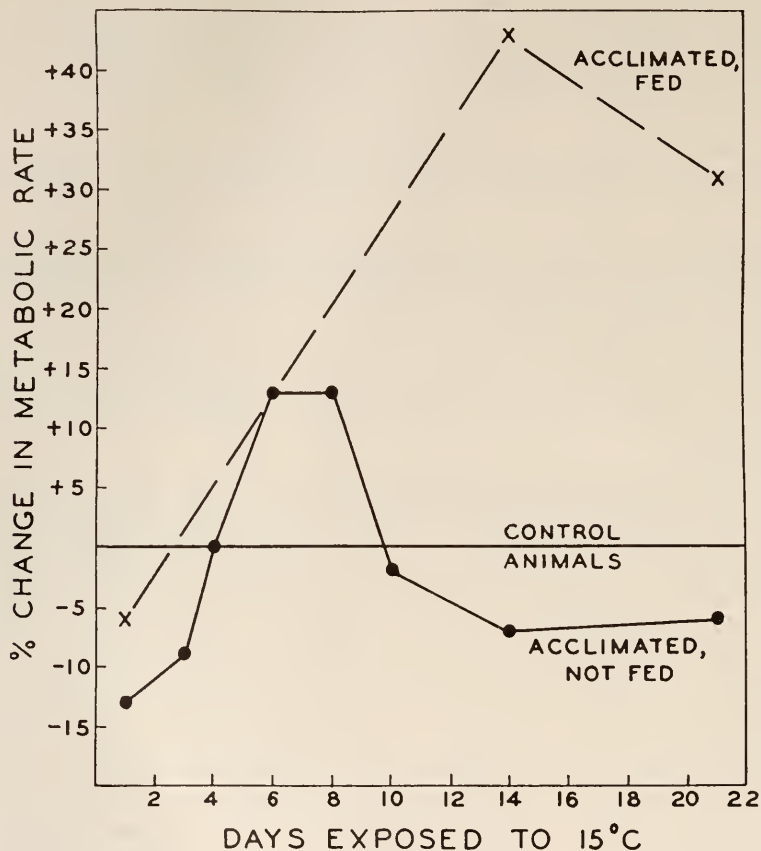

FIGURE 1. The influence of starvation during acclimation to 15° C. on the respiratory metabolism of *Uca pugnax* determined at 27° C. Percentage relationship of changes in rate of oxygen consumption relative to control animals as 0% change.

Their metabolic rate, which was initially depressed by cold, increased by day 6. The rate at day 10 was lower and fluctuated slightly during the remaining 11 days. A tendency to acclimate was apparent until days 6–8 and then was lost.

To determine the role of food in acclimation, this same experiment was repeated with one variation: food was presented to the 15 animals maintained at 15° C. on day 7 and day 11, while the rate of oxygen uptake was determined on days 1, 14, and 21. In a previous paper (Vernberg, 1959), it was shown that their metabolic rate dropped markedly after one day of starvation. This trend

continued until day 3–5 when a leveling-off occurred with minor fluctuations observed until the end of the experiment on day 21. Thus in the present study, it was felt that the metabolic rate determined on day 14 did not reflect post-absorptive effects. Results, which are included in Figure 1, show that food had a pronounced influence on metabolism during temperature acclimation. The metabolic rate did not decline after day 6–8 as observed in the unfed animals, but continued high until the termination of the experiment on day 21.

Table I represents oxygen consumption data on animals which had been acclimated to 15° for various periods of time. The per cent change in metabolism is relative to values obtained for control animals. In this series of determinations, control animals are defined as warm-acclimated animals whose metabolic

TABLE I

The influence of acclimation to 15° C. for various periods of time on the respiratory metabolism of Uca pugnax determined at different temperatures. Percentage relationship of changes in rate of oxygen consumption relative to control animals as 0% change

Temperature ° C.	No. of days at 15°	% change
7	1	+21
7	21	+37*
17	1–2	+24**
17	11	+11
17	21	+16
27	1	−13
27	6	+13
27	14	+43*
33	1	− 8
33	9–11	+ 4
33	18–21	− 3
39	1	− 9
39	11	− 1

* Mean is significantly different from control at 1% level.
** Mean is significantly different from control at 5% level.

rate had been determined at various thermal levels. In all cases, these animals are starved for three to five days before determinations are made. For example, animals acclimated to 15° for 21 days consume oxygen 37% faster than warm-acclimated fiddler crabs when determined at 7° C. A significant difference in metabolic rate during cold acclimation was observed only at a few points. At 7° a marked increase was noted within 24 hours, but it was not until 21 days that the difference in the rate of oxygen consumption was significant at the 1% level. Although individuals exposed for 11 days or 21 days used oxygen at a faster rate than room-animals when determined at 17°, significant differences (at the 5% level) were observed only with 1 day animals. Results obtained at 27° were discussed above. Slight differences were observed at 33° and 39°.

When determined at low temperatures (7° and 17°) the metabolic rate of

cold-acclimated animals was always higher than control animals, but at elevated temperatures (33° and 39°) the reverse was observed (Fig. 2). At an intermediate temperature of 27° the response was intermediate; first the rate was depressed, as was the response at elevated temperatures, and then the rate increased with exposure to cold, much like the response at lower temperatures. The points on Figure 2 for cold-acclimated animals at 7°, 17° and 27° represented the maximum respiratory response to cold.
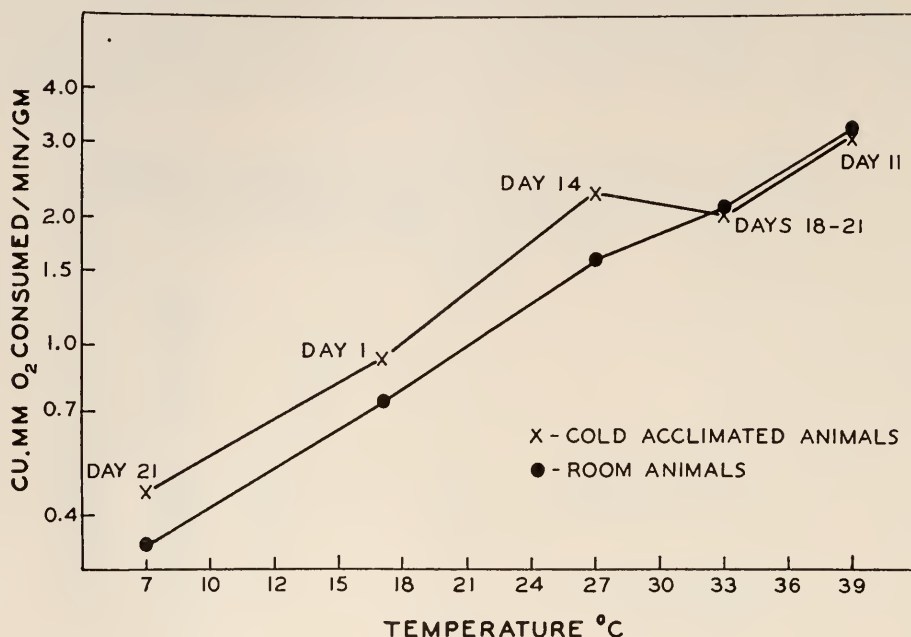

FIIGURE 2. The oxygen consumption at various temperatures of room-animals and cold-acclimated *Uca pugnax* from Beaufort, North Carolina. Cold-acclimated animals had been kept at 15° C. for the periods of time indicated.

Uca rapax from Jamaica

The metabolic rate was determined at 28°, the animals were subjected to 15° for specific intervals of time, and then their metabolic rate determined again at 28°. At the beginning of this experiment 26 animals were used, but this number decreased with time due to death or discarding of animals which had lost appendages: three animals lost their large chela, two animals were dead after three days at 15°, five more had died by the fifth day, and three additional animals had died by day 7. No determinations were made after day 7 as the mortality level was too high. As a more critical method of analysis, the averages for the different days were computed only on the animals surviving throughout a particular time interval, *i.e.,* by day 5 only 18 animals survived, thus the averages cited in Table II reflect the data on only these 18 animals. Results of this experiment show that significant variation was observed only after one-day exposure to 15° and then

only at the 3% level. It should be noted that in Table II the figures for the average metabolic index are not in terms of cu. mm. of O_2/min./gram as were the data on *U. pugnax.* These figures represent the average of the total number of manometer units change divided by the total elapsed time in minutes. For each successive daily determination an animal was placed in the same flask. Thus relative values could be used, and it was not necessary to multiply by the flask constant and divide by the weight of the animal to determine the absolute metabolic rate.

TABLE II

*The influence of acclimation to 15° for various periods of time on the respiratory
metabolism of Uca rapax from Jamaica when determined at 28°*

Number of animals	Number of days at 15°	Average metabolic index	S.E.*
26	0	0.504	0.063
26	1	0.676**	0.048**
23	0	0.518	0.070
23	3	0.544	0.043
18	0	0.504	0.070
18	5	0.577	0.038
13	0	0.484	0.065
13	7	0.523	0.070

* S.E.—Standard Error.
** Means are significantly different at 3% level.

When determining the rate of oxygen consumption at 15° and then after exposure to 15° for one and five days, no significant fluctuation was observed. Similar rates of oxygen uptake determined at 12° were noted for 25 animals kept at room temperature and for 17 animals exposed to 15° for five days. Although temperature acclimation produced no apparent modification in metabolism when determined at low temperatures, marked changes were observed at higher temperatures. After five days at 15°, 19 cold-acclimated crabs consumed oxygen at a significantly higher rate than 26 animals maintained at room temperatures (at the 1% level) when determined at 36° C. These results are graphically represented in Figure 3.

Uca rapax from Alligator Harbor, Florida

Table III presents data on the metabolic response of *Uca rapax* from Alligator Harbor, Florida, after exposure to 15° for various periods of time. Unlike Jamaican forms, but similar to the more northern species, a significant drop (at the 1% level) in metabolic rate resulted after 24 hours at this reduced temperature. By days 3 and 8 no significant difference between non-acclimated and acclimated animals was noted. However, further exposure to cold resulted in a decreased rate which by days 12–14 was significant at the 5% level. Mortality was prac-
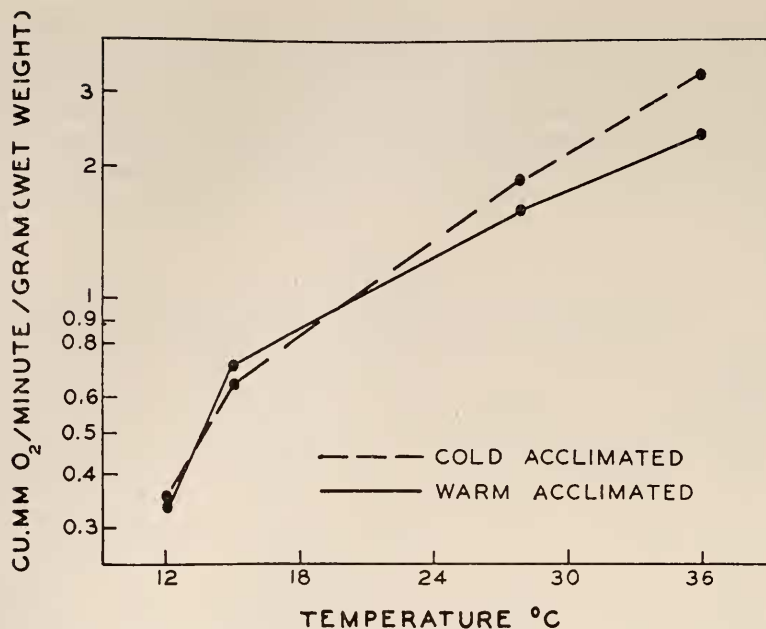
FIGURE 3. The oxygen consumption at various temperatures of warm- and cold-acclimated *Uca rapax* from Jamaica, The West Indies.

TABLE III

Metabolic response of Uca rapax from Alligator Harbor, Florida, after exposure to 15° C. for specific periods of time. Determinations made at various temperatures and results expressed as cu. mm. of O_2 consumed/minute/gram of wet weight

Temperature ° C.	No. days at 15°	Rate of oxygen consumption and standard error	No. of determinations	Significance of difference of means
35	0	2.901 ± 0.149	31	1% level
	11–14	2.026 ± 0.177	16	very significant
28	0	1.916 ± 0.087	58	3% level
28	1	1.695 ± 0.051	58	significant
28	0	1.856 —	36	not significant
28	3	1.666 —	36	
28	0	2.017 ± 0.102	26	not significant
28	8	1.802 ± 0.085	26	
28	0	2.438 ± 0.102	36	5% level
28	12–14	2.144 ± 0.108	36	significant
17	0	1.017 ± 0.066	30	5% level
17	29–53	1.217 ± 0.073	30	significant
7	0	0.352 ± 0.019	24	1% level
7	33–54	0.460 ± 0.011	16	very significant

tically negligible during this two-week period, which is in sharp distinction with the response of tropical forms.

Figure 4 represents the mean metabolic rate of cold-acclimated and room-animals determined at various temperatures. The means are significantly different for these two groups of animals at the four temperature points studied. At 7° and 35°, significance is at the 1% level, while at 17° and 27°–28° significance is at the 5% level. Determinations at 7° and 17° were made on animals maintained at 15° for 29–53 days. No apparent difference was observed in the response of 29 day or 53 day animals; this suggests that acclimation is complete
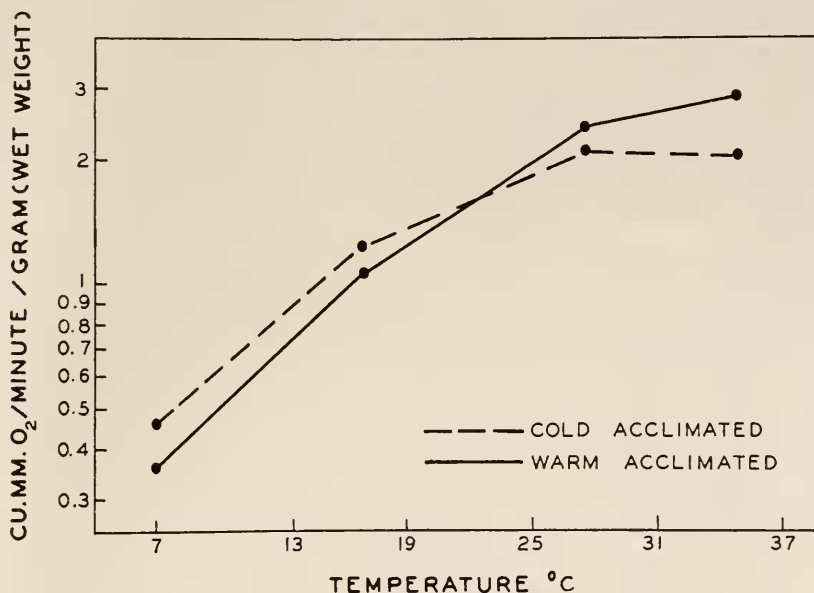

FIGURE 4. The oxygen consumption at various temperatures of warm- and cold-acclimated *Uca rapax* from Alligator Harbor, Florida.

within this time. At 27°–28° and 35°, animals had been kept for 11–14 days. Although greater metabolic differences might have been observed if animals had been maintained at 15° longer, it was felt that on the basis of work on *Uca pugnax* from North Carolina this period of time was sufficiently long to produce marked changes in metabolism.

DISCUSSION

Results of this study on the influence of thermal acclimation on respiratory metabolism furnish further evidence of pronounced physiological differences between temperate and tropical zone fiddler crabs. This work is in general agreement with the findings of other workers in this field.

Roberts (1957), working with population samples of *Pachygrapsus crassipes* collected from southern California to Oregon, found a close correlation between compensatory respiration levels and habitat temperature during the winter. North-

ern crabs consumed oxygen at a faster rate than southern forms when determined at an acclimation temperature higher than the field temperature at the time of collection. Although the experimental conditions in the present study are different from those of Roberts (1957), the same general tendency exists for individuals of the northern population to exhibit higher metabolic rates at low and intermediate temperatures than southern members of the same species. Roberts worked with a species inhabiting the temperate zone and thus subjected to marked seasonal temperature fluctuations, while the *Uca rapax,* used in the present study, extends from the tropics, where the temperature varies slightly during the year, to northern Florida with its seasonal thermal changes. Therefore, it is not surprising to note that the pattern of response of *U. rapax* from Florida is not like the tropical crabs, but more similar to that of *U. pugnax* from North Carolina, especially at temperatures below 17°. It would appear that with a selection pressure for temperature adaptability, a similar metabolic pattern has evolved in temperate zone forms. Although the response of these organisms might be similar, as measured by the oxygen consumption of the whole animal, the mechanisms governing the utilization of oxygen by the cells in these two species may be entirely different.

Uca pugnax appeared to require two weeks to be completely acclimated to low temperature (15°), which shows remarkable similarity to results of Roberts. When comparing these two papers it should be kept in mind that the absolute temperature referred to is not of paramount importance; the relative response of the organisms is the major consideration. In the study of *Pachygrapsus* the temperature (16°) used to base the degree of acclimation to warm or cold was close to the environmental mean for this species. However, in North Carolina the mean habitat temperature of *U. pugnax* during the summer is about 27°.

In both species, *U. pugnax* and *P. crassipes,* cold-acclimated forms consumed oxygen at a faster rate than warm-acclimated animals when determined over a wide temperature range. The only difference was that at elevated temperatures cold-acclimated *U. pugnax* consumed oxygen at the same rate as room-animals, whereas cold-acclimated *P. crassipes* continued to show greater respiratory activity than warm-acclimated forms. Although this difference in response is difficult to explain, it may be associated with the differences in environmental temperature extremes encountered by the two species. At Beaufort, North Carolina, the mean monthly water temperature varies from 5.5° in February to 28° in August (McDougall, 1943), while data presented by Roberts show yearly changes of a low of about 13° to a high of about 18° for Point Hueneme, California.

Working with *U. pugilator* from Massachusetts and Florida, Démeusy (1957) measured their respiratory rates at 1.4° and 15° after being maintained at 20° for various time intervals. The northern forms showed higher rates throughout a seven-week period when determined at 1.4°. Although no statistically significant difference was noted between the respiratory activity of the two groups of fiddler crabs at 15°, it should be noted that determinations were not made at this temperature over an extended period of time. In the present study, northern forms of *U. rapax* consumed oxygen at a higher rate than southern forms, especially at lower temperatures, which is similar to the findings of both Démeusy and Roberts,

In contrast to the results of Roberts, Démeusy found no decrease in metabolic rate of crabs from different localities when kept at a common temperature. She felt that regular feeding of the animals might be responsible for this steady rate of metabolism. Results of the present paper demonstrate the following: (1) feeding of *U. pugnax* during acclimation to 15° resulted in the maintenance of a steady rate of oxygen consumption after two weeks; and (2) the temperate zone forms of *U. rapax* showed a gradual decline in metabolic rate during acclimation to 15°, as shown by Roberts, while the tropical animals showed a tendency to have higher rates.

The respiratory activity at 1.4° of *U. pugilator* from Florida decreased slightly for the first seven days of acclimation to 10° when compared with crabs kept at room temperature. This decrease was followed by a slight increase after one week. Although *U. rapax* from Florida and *U. pugnax* were subjected to slightly different experimental conditions, cold-acclimated animals of both species consumed oxygen at significantly higher levels than room-animals when determined at 7°. This response is similar to findings of Roberts on *Pachygrapsus*. However, the respiratory pattern of the tropical form of *U. rapax* did not change during acclimation to 15° except at elevated temperatures.

Recently Teal (1959) reported that on the basis of studies on the crabs found in one area, a Georgia salt marsh, *U. pugnax* had a better developed mechanism for thermal acclimation than *U. pugilator*. This might help explain some apparent differences between the results of Démeusy and those of the present paper.

Prosser (1958) has described various patterns of temperature acclimation based on measuring reaction rates over a range extending beyond the acclimation temperature. Using his terminology, it is possible to characterize the pattern of response of the tropical and temperate zone fiddler crabs used in the present study.

Tropical forms of *U. rapax* appear to best fit pattern I (no adaptation) except between 28° and 36° where the Q_{10} of cold-acclimated animals is increased significantly over that of warm-acclimated organisms, suggesting pattern IIIB. Similar results have been reported by Scholander *et al.* (1953), when comparing some tropical and arctic terrestrial insects. The metabolic response of the temperate zone form of *U. rapax* appears to resemble pattern IIIA, which indicates that the Q_{10} of cold-acclimated animals is less than the Q_{10} of warm-acclimated forms. Démeusy (1957) found this response in *U. pugilator*. *U. pugnax* from North Carolina seems to fit pattern IVA best, which seems to be the most common method of temperature acclimation, according to Prosser. Rao (1953) observed this pattern in the rate of pumping water by *Mytilus* from different latitudes.

On the basis of this method of comparing patterns of metabolic response during temperature acclimation, certain generalizations might be made. Both populations of *U. rapax* demonstrated a rotation of rate function curves, whereas in *U. pugnax* the type of change is a translation of curves. This might suggest that different biochemical mechanisms are involved in these two species.

The results of Tashian (1956) on oxygen consumption of *Uca* from different latitudes indicate that different patterns may be found when making intra- and inter-specific comparisons of animals from different geographic locations. This was noted also in the present study. Intraspecific comparisons of *U. pugnax*

from New York and North Carolina and *U. rapax* from Florida and Trinidad represent pattern IIA. An inter-specific comparison of *U. pugnax* from New York and *U. rapax* from Trinidad and Florida suggests pattern IIIA, while comparing *U. pugnax* from North Carolina and *U. rapax* from Florida seems to indicate pattern IVB. It should be noted that this work was done on animals with similar recent thermal histories and determinations were made at two temperatures. Vernberg (1959) measured the rate of oxygen consumption over a wide temperature range of 7 species of temperate and tropical zone fiddler crabs. He noted that systematic differences correlated with latitude were found only at lower temperatures. The findings of the present paper, which involved acclimation studies, suggest that with the evolution and distribution of animals to new environmental complexes a new pattern of acclimation has resulted.

Summary

1. Studies on the metabolic response of tropical and temperate zone fiddler crabs (Genus *Uca*), after various periods of thermal acclimation, were done to assess the importance of this factor in the evolution and climatic adaptation of these forms.

2. *U. pugnax,* a temperate zone species, showed a significantly higher metabolic rate after acclimation to 15° than warm-acclimated forms when determined at 27°, 17° and 7°, but not at 33° and 39°. When animals were starved during the period of acclimation, the tendency to acclimate was apparent by days 6–8 and then was lost. Feeding maintained the pattern of acclimation for at least 21 days.

3. *U. rapax* from Jamaica, The West Indies, did not show any shift in metabolic response during acclimation to 15°, except when determined at 36°, where the rate of cold-acclimated forms was higher than warm-acclimated animals.

4. Population samples of *U. rapax* from Florida responded metabolically like temperate zone animals at low temperatures and the tropical animals at high temperatures.

5. Results of this study demonstrated that during the evolution and distribution of fiddler crabs different patterns of acclimation have resulted.

LITERATURE CITED

Bullock, T. H., 1955. Compensation for temperature in the metabolism and activity of poikilotherms. *Biol. Rev.,* **30**: 311–342.

Démeusy, N., 1957. Respiratory metabolism of the fiddler crab *Uca pugilator* from two different latitudinal populations. *Biol. Bull.,* **113**: 245–253.

McDougall, K. D., 1943. Sessile marine invertebrates at Beaufort, North Carolina. *Ecol. Monog.,* **13**: 321–374.

Prosser, C. Ladd, 1955. Physiological variation in animals. *Biol. Rev.,* **30**: 229–262.

Prosser, C. Ladd, ed., 1958. Physiological Adaptation. American Physiological Society, Washington, D. C.

Rao, K. P., 1953. Rate of water propulsion in *Mytilus californianus* as a function of latitude. *Biol. Bull.,* **104**: 171–181.

Roberts, J. L., 1957. Thermal acclimation of metabolism in the crab *Pachygrapsus crassipes* Randall. II. Mechanisms and the influence of season and latitude. *Physiol. Zoöl.,* **30**: 242–255.

Scholander, P. F., W. Flagg, V. Walters and L. Irving, 1953. Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zoöl.,* **26**: 67–92.

TASHIAN, R. E., 1956. Geographic variation in the respiratory metabolism and temperature coefficient in tropical and temperate forms of the fiddler crab, *Uca pugnax*. *Zoologica,* **41** : 39–47.

TASHIAN, R. E., AND F. J. VERNBERG, 1958. The specific distinctness of the fiddler crabs *Uca pugnax* (Smith) and *Uca rapax* (Smith) at their zone of overlap in northeastern Florida. *Zoologica,* **43** : 89–93.

TEAL, J. M., 1959. Respiration of crabs in Georgia salt marshes and its relation to their ecology. *Physiol. Zoöl.,* **32** : 1–14.

VERNBERG, F. J., AND R. E. TASHIAN, 1959. Studies on the physiological variation between tropical and temperature zone fiddler crabs of the genus *Uca*. I. Thermal death limits. *Ecology* (in press).

VERNBERG, F. J., 1959. Studies on the physiological variation between tropical and temperature zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *Biol. Bull.,* **117** : 163–184.